

# Comparison of a Nerve Gas Detoxifying Enzyme from Squid and from *Pseudomonas diminuta*

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Potent acetylcholinesterase (AChE) inhibitors such as DFP, (iC<sub>3</sub>H<sub>7</sub>O)<sub>2</sub>P(=O)F, and Soman, CH<sub>3</sub>(C<sub>6</sub>H<sub>13</sub>O)P(=O)F, are hydrolyzed and detoxified by an enzyme in the nerve tissue of squid and other cephalopods (1). A second generation of these highly toxic compounds, typified by VX, CH<sub>3</sub>(C<sub>2</sub>H<sub>5</sub>O)P(=O)SCH<sub>2</sub>CH<sub>2</sub>N(iC<sub>3</sub>H<sub>7</sub>)<sub>2</sub>, and Tetriso, (iC<sub>3</sub>H<sub>7</sub>O)<sub>2</sub>P(=O)SCH<sub>2</sub>CH<sub>2</sub>N(iC<sub>3</sub>H<sub>7</sub>)<sub>2</sub>, are not hydrolyzed by the Soman-hydrolyzing enzyme from squid, termed “squid type” organophosphorus acid anhydrolase (OPAA). Recently we reported an organophosphorus hydrolase (OPH) from *Pseudomonas diminuta* that hydrolyzes VX and Tetriso, and also DFP and Soman (2). Soman and VX are major components of now unwanted stockpiles of the so-called “nerve gases” (3).

Although squid type OPAA will not hydrolyze VX or Tetriso, these compounds may occupy the active site of the squid enzyme and thus inhibit its hydrolysis of DFP or Soman. Similarly, although the *Pseudomonas* OPH hydrolyzes all four compounds, VX or Tetriso might inhibit the hydrolysis of DFP or Soman, or DFP or Soman might inhibit the hydrolysis of VX or Tetriso. While the findings of such enzymes, seemingly without natural substrates, pose interesting fundamental questions that may be explored by combinations of these potential substrates and inhibitors, the disposal of mixed stockpiles of these nerve gases, as for example in the Gulf War aftermath, also poses practical questions.

Although Soman and VX are not readily available, findings with DFP and Tetriso are applicable to the nerve gas combinations, *i.e.*, Soman and VX. To these ends, we have determined the enzymatic hydrolysis rates of DFP (and in a few experiments, Soman) in the presence and absence of Tetriso, and of Tetriso in the presence and absence of DFP (and in a few experiments, Soman). This is possible because the hydrolysis of DFP and Soman can be determined with a fluoride-sensitive electrode (1) even in the presence of Tetriso, and the hydrolysis of Tetriso can be determined with DTNB by the “direct” Ellman reaction (2, 4), even in the presence of DFP or Soman.

In a typical experiment of the first kind, a fluoride-sensitive electrode was placed in a stirred solution of 1.9 ml 0.025 M Hepes buffer (pH 7), 1.5 ml 0.01 M Tetriso, and 1.5 ml 0.01 M DFP. After determining the non-enzymatic rate, 0.1 ml OPH enzyme was added. The ensuing fluoride release was compared to an identical reaction in which Tetriso was replaced by buffer. In a typical experiment of the second kind, buffer, Tetriso, and DFP (1.06 ml of each), and 0.2 ml DTNB (1) were mixed in two cuvettes. Absorbancy at 412 nm was noted (zero during 5 min), 0.1 ml OPH was added to the reaction vessel, and readings were continued. The absorbancy was compared to an identical reaction in which DFP was replaced by buffer.

**Table I**

*Enzymatic hydrolysis of organophosphorus nerve gases singly and in pairs*

Substrate (inhibitor)	Activity (% inhibition) of	
	OPH from <i>Pseudomonas diminuta</i>	OPAA from Squid ( <i>Loligo pealei</i> ) nerve
DFP	100 <sup>1</sup>	100 <sup>1</sup>
DFP (Tetriso)	87 (13%)	96 (4%)
Soman	31	26
Soman (Tetriso)	29 (6%)	26 (0%)
Tetriso	0.53	0 <sup>2</sup>
Tetriso (DFP)	0.02 (96%)	Not measurable
Tetriso (Soman)	0.36 (32%)	Not measurable

<sup>1</sup> Arbitrarily set at 100.

<sup>2</sup> No readable results, even with a 50-fold excess of enzyme.

The results of triplicate determinations (std. dev., <5%) are given in Table I. Because the data are electrode and spectrophotometer readings, and because the two enzyme sources were purified to different degrees, always with respect to DFP as substrate, those results (line 1 of Table I) were set to 100. All of the *Pseudomonas* readings were significantly greater than zero by the expedient of increasing the amount of enzyme, increasing the instrument sensitivity, or both; but with Tetriso in the role of substrate, a 50-fold increase of the amount of OPAA over that used with DFP, and full-scale absorbancy set at 0 to 0.1, there was still no evidence of Tetriso hydrolysis by the squid enzyme.

The results in Table I were obtained with  $3 \times 10^{-3}$  M DFP, or Soman, or Tetriso, whether singly or in combination. They suggest that the affinity of the active site of OPH is greater for DFP, and probably Soman, than for Tetriso and, by analogy, VX. To explore this notion with the *Pseudomonas diminuta* OPH only, activities were determined over the concentration range of  $5 \times 10^{-3}$  to  $10^{-4}$  M. For Tetriso as substrate,  $K_M \approx 5 \times 10^{-3}$  M; with DFP as the potential inhibitor,  $K_i \approx 2 \times 10^{-4}$  M. In the reverse experiment, with DFP as substrate,  $K_M \approx 8 \times 10^{-5}$  M, and with Tetriso as inhibitor,  $K_i \approx 3 \times 10^{-3}$  M. The inhibitions appear to be competitive but the concentration ranges, dictated by solubilities and the sensitivities of the methods, make both the constants and the nature of the inhibitions subject to some uncertainty. At a fundamental level, the active site of the *Pseudomonas* OPH appears to bind the P-F compounds about 50 times more strongly than the P-S-alkylamino group. The squid OPAA does not bind this latter group at all. At a practical level, these results suggest that additional hazards may be encountered in attempting to use enzymatic means for the disposal of mixed nerve gas stockpiles (3).

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## Literature Cited

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Aspects of Lead Toxicity and Habituation in *Hermisenda* Learning

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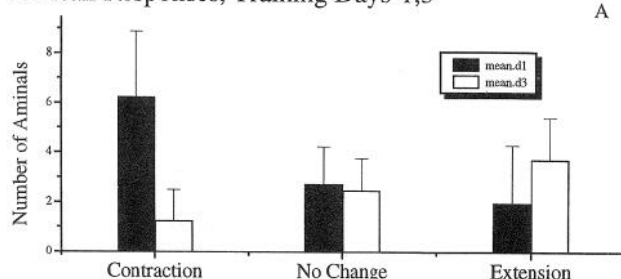
Lead toxicity remains a threat to human health and cognitive development (1). Investigating this multifaceted problem is difficult because lead lacks a single definable mechanism of action. The multiplicity of action sites confounds the straightforward

development of a single hypothesis to explain all the reported data (2, 3, 4).

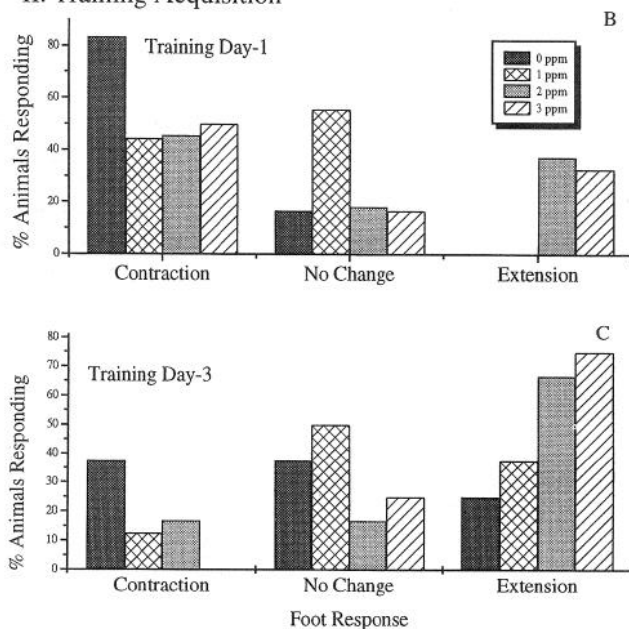
*Hermisenda* has served as a neurobiological model for learning and memory studies for over 25 years. Many of the physiological properties underlying the behavioral modification this marine mollusc undergoes as a result of associative (Pavlovian) conditioning have been elucidated (5, 6). In an effort to demonstrate that this animal can be an effective model system for studying lead toxicity, we are testing various aspects of lead on the behavior and learning capabilities of *Hermisenda*. This report includes data on how lead exposure affects training acquisition and habituation by *Hermisenda*. It represents a portion of the data supporting the hypothesis that the behavioral alterations measured in lead-exposed animals reflect alterations in central nervous system processing and are unlikely to be caused by some general physiological impairment or loss of motor ability.

Animals (Sea Life Supply, Sand City, CA) were received

## I. Mean Responses, Training Days-1,3



## II. Training Acquisition



**Figure 1.** (A) I. Mean responses, training Days-1, 3: Graph depicts the mean responses of all animals to agitation on training Day-1 and Day-3. Irrespective of lead exposure (data pooled for lack of treatment effects), agitation elicited greater positive responses on Day-1; by Day-3, significant evidence of habituation was present. However, when these same animals were tested on Day-4 as per standard operating procedures, 78% of the control animals contracted with light alone, but only 33% of the 3 ppm PbAc-exposed animals contracted (normal data; see Kuzirian et al. (10)). (B–C) II. Training acquisition: Graphs illustrate the degree of response to 5 trials of paired light/agitation incurred by animals exposed to 0, 1, 2, and 3 ppm PbAc at the beginning of training Day-1, and Day-3. On training Day-1 (B), 83% of the 0 ppm PbAc controls contracted, whereas none of that group or the 1 ppm lead-exposed animals lengthened the foot in normal locomotion. Conversely, on training Day-3 (C), the percentage of control animals that exhibited a positive response dropped to 37.5% but the percentage of those animals extending the foot in response to agitation increased to 25%. Animals with higher lead exposures continued their trend of foot extension, raising the response rate to 66%–75%. The phenomenon demonstrated is habituation to the unconditioned stimulus (UCS), agitation. The habituation response was greater in the animals exposed to the higher concentrations of lead.